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A nanofluidic device for mapping single DNA molecules to the human genome

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Single DNA molecules can be studied using nanofluidics. Individual DNA molecules of genomic length can be stretched by confinement in nanochannels. This has been used to characterize the base pair sequence [1-3], or the methylation [4] of DNA by imaging fluorescence barcodes of single molecules stretched in nanochannels. In nanochannel devices, DNA stretching is provided by confinement only i.e. DNA can be fully stretched if the channel cross-section matches the persistence length of the DNA. This requires working at low ionic strength [5] or fabricating nanochannels using sub-100 nm lithography. If not, the incomplete stretching of DNA results in longitudinal motion of the DNA molecule excited by thermal noise that blurs the fluorescence barcode. There is thus a need for a modified device architecture providing high stretching in any buffer conditions and allowing a simple fabrication scheme.

We address this challenge by designing a nanofluidic device where the stretching of genomic DNA is achieved by an additional mechanism: the hydrodynamic drag of a buffer flow [6] (figure 1). Hydrodynamic drag in a cross-shaped microchannels has previously been used to stretch viral DNA at the stagnation point where molecules were imaged before they would escape [7]. Our device architecture based on a cross-shaped nanoslit immobilizes long DNA fragments (>440 μm) by providing the initial stretching in a 85 nm-high slit (figure 1C). At the onset of the shear flow in the nanoslit, a hydrodynamic drag pulls on the DNA in opposite directions (figure 1A-B) and increases the stretching to 98%. At such high stretching, the number of base pairs included in the diffraction limit is minimized thus providing the best barcode resolution obtainable using conventional epifluorescence (about 1 kilobase). The device requires no sub-micron lithography and allows stretching DNA at a wide range of buffer conditions.

We used our device to image fluorescence barcodes generated on mega base pair long human DNA. Human genomic DNA was obtained by proteolysis of metaphase chromosomes directly in the inlet wells of the device (figure 2A). The barcode was obtained by staining the DNA homogeneously with YOYO-1 followed by a thermal partial denaturation of the DNA and renaturation (figure 2B). This creates a stable fluorescence pattern specific to the underlying base sequence. After stretching in the device (figure 2C), the fluorescence barcode of individual molecules is recorded, covering a minimum of 1.4 mega base pairs (figure 2D). We have shown that the barcode image enables to map each fragment to its origin in the human reference genome (figure 2E). Moreover, we were able to detect large structural variations (from a couple of kilobase and up) present in single copies of the human genome by comparing the fluorescence pattern of a given molecule to the pattern expected from the human reference genome (hg18). We have thus shown that using a device architecture combining confinement and hydrodynamic drag, full stretching of DNA molecules can be obtained and that in return, the high barcode resolution could be used to characterize genome structural variations on a single molecule level.

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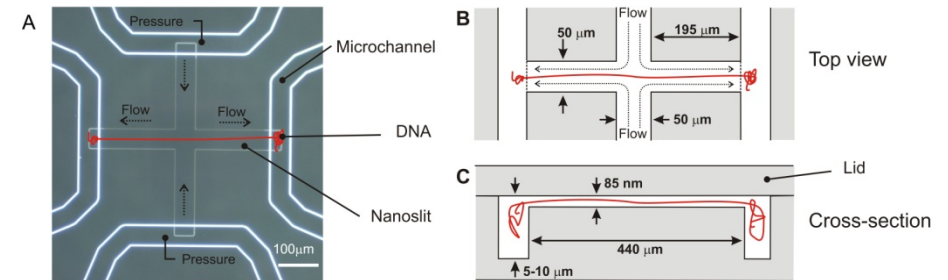


Figure 1. (A) Optical image of the silica nanofluidic device showing a cross-shaped nanoslit used to stretch single DNA fragments (sketched) to almost full contour length. The liquid flow in the nanoslit (arrows) pulls symmetrically on the DNA. (B) Top and (C) cross section view of the device geometry.

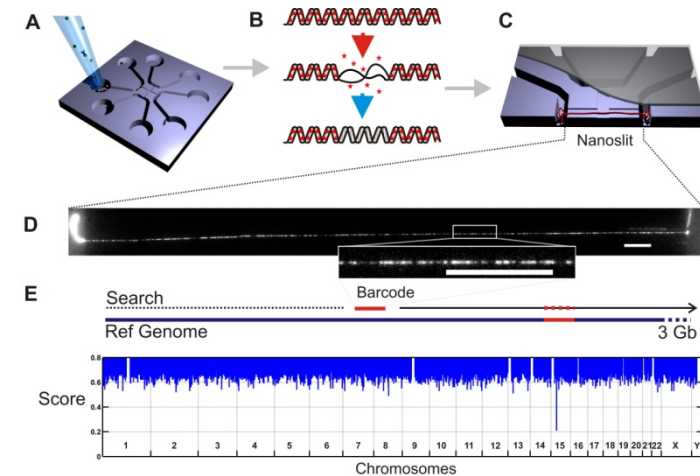


Figure 2. The fluorescence denaturation-renaturation map of a 1.4×10^6 base-pairs long DNA molecule extracted from human metaphase chromosomes (scale bar is $20 \mu\text{m}$). A shorter piece of the barcode (125kb) is used to map the molecule to its location on chromosome 15 of the human reference genome (a total of 3×10^9 bases).